PATENT

Attorney's Docket No.: U 012254-3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of Inventors:

1. RAM **PRATAP**
2. AMIYA PRASAD **BHADURI**
3. HARSH PATI **THAPLIYAL**
4. SUNIL KUMAR **PURI**
5. GURU PRASAD **DUTTA**
6. ANIL KUMAR **DWIVEDI**
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11. SHIO KUMAR **SINGH**
12. RAM CHANDRA **GUPTA**
13. JAGDISHWAR SAHAI **SRIVASTAVA**
14. OMKAR PRASAD **ASTHANA**

WARNING: *The Declaration must name all of the actual inventor(s).*

For (title):

METHOD FOR THE TREATMENT OF MALARIA BY THE USE OF PRIMAQUINE DERIVATIVE N^1-(3-ETHYLIDINOTETRAHYDROFURAN-2-ONE)-N^4- (6-METHOXY 8-QUINOLINYL)-1,4-PENTANEDIAMINE AS GAMETOCYTOCIDAL AGENT

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this New Application Transmittal and the documents referred to as enclosed therein are being deposited with the United States Postal Service on this date **MAY 21, 1999 in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EE784099332US addressed to the: Assistant Commissioner of Patents, Washington, D.C. 20231**

CONNIE YANNOTTI
(type or print name of person mailing paper)

(Signature of person mailing paper)

NOTE: ***Each paper or fee referred to as enclosed herein has the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 CFR 1.10(b).***

WARNING: ***Certificate of mailing (first class) or facsimile transmission procedures of 37 CFR 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.***

EE784099332US

1. Type of Application

This new application is for a(n) (check one applicable item below):

- ☑ Original (nonprovisional)
- ☐ Design
- ☐ Plant

WARNING: ***Do not*** *use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4) unless the International Application is being filed as a divisional, continuation or continuation-in-part application.*

WARNING: ***Do not*** *use this transmittal for the filing of a provisional application.*

2. Benefit of Prior U.S. Application(s) (35 U.S.C. 119(e), 120, or 121)

NOTE: If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.*

WARNING: *When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional* ***must*** *be filed prior to the Saturday, Sunday or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3).*

- ☐ The new application being transmitted claims the benefit of prior U.S. application(s) and enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

NOTE: If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION.

- ☐ Divisional.
- ☐ Continuation.
- ☐ Continuation-in-Part (C-I-P).

3. Papers Enclosed That Are Required For Filing Date Under 37 CFR 1.53 (Regular) or 37 CFR 1.153 (Design) Application

- 23 Pages of specification
- 2 Pages of claims
- 1 Pages of Abstract
- _ Sheets of drawing
  - ☐ formal
  - ☐ informal

WARNING: ***DO NOT*** *submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. Comments on proposed new 37 CFR 1.84. Notice of March 9, 1988 (1990 O.G. 57-62).*

NOTE: "Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (⅝ inch) down from the top of the page." 37 C.F.R. 1.84(c).

(complete the following, if applicable)

☐ The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)". 37 C.F.R. 1.84(b).

4. Additional papers enclosed

☐ Preliminary Amendment

☐ Information Disclosure Statement (37 CFR 1.98)

☐ Form PTO-1449

☐ Citations

☐ Declaration of Biological Deposit

☐ Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.

☐ Authorization of Attorney(s) to Accept and Follow Instructions from Representative

☐ Special Comments

☐ Other

5. Declaration or oath

☐ Enclosed

executed by *(check **all** applicable boxes)*

☐ inventors.

☐ legal representative of inventors. 37 CFR 1.42 or 1.43

☐ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.

☐ This is the petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 is also attached. *See item 13 below for fee.*

☑ Not Enclosed.

WARNING: *Where the filing is a completion in the U.S. of an International Application but where a declaration is not available or where the completion of the U.S. application contains subject matter in addition to the International Application the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.*

☑ Application is made by a person authorized under 37 CFR 1.41(c) on behalf of *all the above named inventors.* (The declaration or oath, along with the surcharge required by 37 CFR 1.16(e) can be filed subsequently).

NOTE: It is important that all the correct inventor(s) are named for filing under 37 CFR 1.41(c) and 1.53(b).

☐ Showing that the filing is authorized. *(Not required unless called into question. 37 CFR 1.41(d).)*

6. Inventorship Statement

WARNING: *If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.*

The inventorship for all the claims in this application are:

☐ The same

☐ Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. A verified English translation of the non-English language application and the processing fee of $130.00 required by 37 CFR 1.17(k) is required to be filed with the application or within such time as may be set by the Office. 37 CFR 1.52(d).

NOTE: A non-English oath or declaration in the form provided or approved by the PTO need not be translated. 37 CFR 1.69(b).

☑ English

☐ non-English

☐ the attached translation is a verified translation. 37 CFR 1.52(d).

8. Assignment

☑ An assignment of the invention to COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH

☐ is attached. A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

☑ will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters—one for the application and one for the assignment." Notice of May 4, 1990 (1114 O.G. 77-78).

WARNING: *A newly executed "CERTIFICATE UNDER 37 CFR 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993. 1150 O.G. 62-64.*

9. Certified Copy

Certified copy of application

Country	Appln. No.	Filed
India	NOT YET KNOWN	April 29, 1999

from which priority is claimed

☐ is attached.

☑ will follow.

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration. 37 CFR 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself

entitled to priority from a prior foreign application then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. Fee Calculation (37 CFR 1.16)

A. ☑ Regular Application

Claims as Filed								
Number Filed			**Number Extra**			**Rate**	**Basic Fee 37 CFR 1.16(a) $760.00**
Total Claims (37 CFR 1.16(c))	10	- 20 =	0	x	$	18.00	
Independent Claims (37 CFR 1.16(b))	3	- 3 =	0	x	$	78.00	
Multiple dependent claim(s), if any (37 CFR 1.16(d))				+	$	260.00	

☐ Amendment cancelling extra claims enclosed.

☐ Amendment deleting multiple-dependencies enclosed.

☐ Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 CFR 1.16(d).

Filing Fee Calculation $

B. ☐ Design application ($310.00 — 37 CFR 1.16(f))

Filing Fee Calculation $

C. ☐ Plant application ($480.00 — 37 CFR 1.16(g))

Filing Fee Calculation $

11. Small Entity Statement(s)

☐ Verified Statement(s) that this is a filing by a small entity under 37 CFR 1.9 and 1.27 is(are) attached or has been filed.

Filing Fee Calculation (50% of **A**, **B** or **C** above) $

NOTE: Any excess of the full fee paid will be refunded if a verified statement and a refund request are filed within 2 months of the date of timely payment of a full fee. 37 CFR 1.28(a).

12. Request for International-Type Search (37 CFR 1.104(d)) *(Complete, if applicable)*

☐ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made At This Time

☑ Not Enclosed

☑ No filing fee is to be paid at this time. *(This and the surcharge required by 37 CFR 1.16(e) can be paid subsequently.)*

☐ Enclosed

☐ basic filing fee $

☐ Recording assignment ($40.00; 37 CFR 1.21(h)) (See attached "COVER SHEET FOR ASSIGNMENT ACCOMPANYING NEW APPLICATION.")

☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached. ($130.00; 37 CFR 1.47 and 1.17(h)) $

☐ For processing an application with a specification in a non-English language. ($130.00; 37 CFR 1.52(d) and 1.17(k)) $

☐ Processing and retention fee ($130.00; 37 CFR 1.53(d) and 1.21(l))

☐ Fee for international-type search report ($40.00; 37 CFR 1.21(e)). $

NOTE: 37 CFR 1.21(l) establishes a fee for processing and retaining any application which is abandoned for failing to complete the application pursuant to 37 CFR 1.53(d) and this, as well as the changes to 37 CFR 1.53 and 1.78, indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid or the processing and retention fee of §1.21(l) must be paid within 1 year from notification under §53(d).

Total fees enclosed $

14. Method of Payment of Fees

☐ Check in the amount of $

☐ Charge Account No. 12-0425 in the amount of $

A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 CFR 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: *If no fees are to be paid on filing, the following items should <u>not</u> be completed.*

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.*

☐ The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No. 12-0425.

☐ 37 CFR 1.16(a), (f) or (g) (filing fees)

☐ 37 CFR 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

☐ 37 CFR 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

☐ 37 CFR 1.17 (application processing fees)

WARNING: *While 37 CFR 1.17(a), (b), (c) and (d) deal with extensions of time under §1.136(a), this authorization should be made only with the knowledge that: "Submission of the appropriate extension fee under 37 C.F.R. 1.136(a) is to no avail unless a request or petition for extension is filed." (Emphasis added). Notice of November 5,1985 (1060 O.G. 27)*

☐ 37 CFR 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 CFR 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 CFR 1.311(b).

NOTE: 37 CFR 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application ... prior to paying, or at the time of paying, ... issue fee". From the wording of 37 CFR 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions As To Overpayment

☐ credit Account No. 12-0425

☐ refund

Signature of Attorney

Reg. No.

Tel. No.

WILLIAM R. EVANS
c/o LADAS & PARRY
26 WEST 61st STREET
NEW YORK, N.Y. 10023
Reg. No. 25,858 (212) 708 1945

☐ **Incorporation by reference of added pages**

(Check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

☐ Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added ___

☐ Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added ___

☐ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added ___

☑ **Statement Where No Further Pages Added**

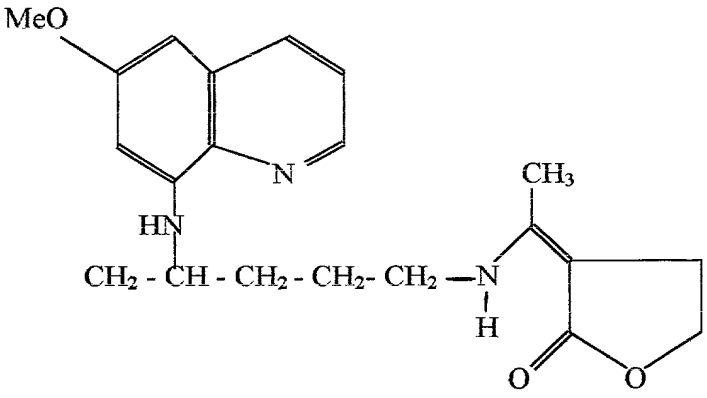
(If no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item:)

☑ This transmittal ends with this page.

Method for the treatment of malaria by the use of Primaquine derivative N^1-(3-ethylidinotetrahydrofuran-2-one)-N^4- (6-methoxy-8-quinolinyl)-1,4-pentanediamine as Gametocytocidal agent

Field of the invention:

The present invention relates to a method of treatment of malaria by the use of primaquine derivative N^1 - (3-ethylidinotetrahydrofuran-2-one)-N^4 - (6-methoxy-8-quinolinyl)-1,4-pentanediamine as a gametocytocidal agent. More particularly, this invention relates to the use of primaquine derivative N^1- (3-ethylidinotetrahydrofuran-2-one)-N^4- (6-methoxy-8-quinolinyl)-1,4-pentanediamine of formula 1 shown below useful for controlling the spread of malaria by virtue of its high therapeutic value as a gametocytocidal agent.



(1)

The primaquine derivative of the present invention does not damage either normal or G-6PD deficient erythrocytes to the extent it is observed with the use of primaquine.

Background of the invention:

Malaria is one of the most serious protozoal infections in man. According to estimation made in the 90's, about 300 to 500 million people develop clinical infection and one million die of severe infection every year. India is also among the countries to have endemic regions of the disease. It is, therefore, of prime concern and requirement to have therapeutically safe agents for multiple use, especially those that block transmission of malaria through the individuals visiting endemic regions. A recent report of resurgence of malaria after a long gap of 40 years from Italy through transmission, highlights our concern [The Lancet, 350, 717 (1997)].

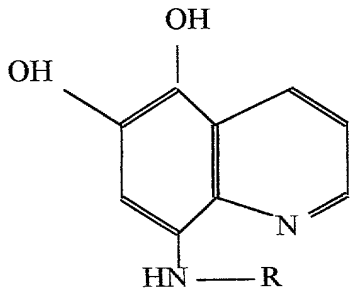
Malaria is caused by infection with any one of the four species of *Plasmodia*. The life cycle of *Plasmodia* is complex and comprises a sexual phase (called sporogyny) in the mosquito (a vector) and an asexual division (called schizogyny) in humans. The life cycle starts after injection of sporozoites by the bite of an infected female *anopheline* mosquito. Sporozoites then rapidly enter into liver parenchymal cells where they undergo exoerythrocytic schizogony forming exoerythrocytic stage of tissue schizonts which mature and release thousands of merozoites in the bloodstream upon the rupture of infected cell. Some of these merozoites enter erythrocytes where they transform into trophozoites and schizonts. The mature schizonts rupture and release merozoites into the circulation, which can infect other erythrocytes. This is termed as asexual schizogony (erythrocytic cycle) and it is this periodic release of merozoites which is responsible for characteristic periodicity of the fever in malaria. After several erythrocytic cycles, some erythrocytic forms differentiate into sexual forms called gametocytes. In *P. vivax* and *P. ovale* infections, some of the sporozoites after entering the liver cells are known to remain dormant and form the latent tissue stage called hypnozoites. These hypnozoites upon activation develop secondary tissue schizonts, which are responsible for the recurrence of malaria called relapsing malaria. The 8- aminoquinoline antimalarial drugs of which primaquine (PQ) is of exceptional importance, have been demonstrated to possess activity against several life cycle stages of the parasite. These agents are active against the primary tissue schizonts, thus functioning as causal-prophylactic agents, against the secondary exoerythrocytic forms and curing relapsing forms of malaria. The transmission of malaria as discussed earlier, is through the injection of sporozoites by the bite of mosquitoes. These sporozoites develop in the mosquito feeding on an individual carrying mature gametocytes. The male and female gametocytes upon ingestion by a female anopheline mosquito fertilize and transform into zygote and ookinete stages. The ookinetes pierce through the epithelium of the midgut where it rounds up into the oocyst. A single oocyst contains as many as 10000 sporozoites. Primaquine has no sporontocidal activity when provided directly to the insects but has strong gametocytocidal activity and even stops transmission of resistant isolates when mosquitoes are fed on infected blood from primaquine treated animals. Thus, primaquine is also a strong transmission blocking agent. However, primaquine even being associated with radical curative and gametocytocidal activities is not in use as a prophylactic agent.

The practical problems associated with use of 8- aminoquinolines are mainly related to their toxicity because of prolonged use in radical treatment required due to fast metabolism of the drug. Primaquine is known to induce hemolytic lesions in patients suffering from a

deficiency in glucose-6- phosphate-dehydrogenase (G-6PD), a genetic condition common among inhabitants in regions where malaria is endemic. Anemia is a common complication of hemolysis. Primaquine produces metabolites like o-quinone and p-quinomine functionalities, which because of their oxidative nature, oxidise unsaturated fatty acid of erythrocytes causing red blood cell (RBC) lysis. The reduced glutathione (GSH) controls the level of oxidative metabolites and the level of GSH is maintained through NADPH controlled GSSG reduction. NADPH is regulated by G-6PD and hence G-6PD deficient patients are more liable to RBC lysis. Primaquine is the only antimalarial drug, which inhibits the development of the parasite by interfering at several stages of the parasitic life cycle and therefore an ideal molecule for structural modification to provide a molecule with radical curative and gametocytocidal activities with low toxicity. The study of the fate of primaquine, its metabolites and toxic manifestation in relation with metabolites will therefore, guide the direction of changes in the new molecule. A brief discussion of primaquine metabolism is given here.

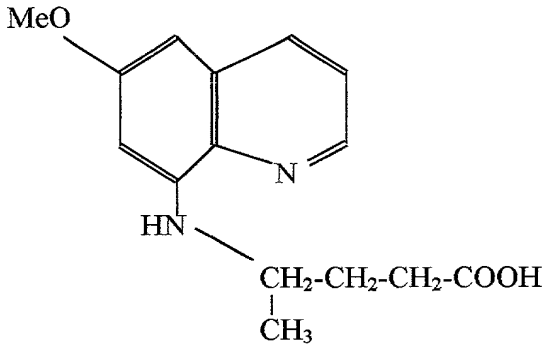
Following oral administration of labelled primaquine it was found that 45 % of the radioactivity was found in liver tissue, and 22 % in the lung, adrenal, spleen, kidney, heart, blood and pancreas while 25 % reached in to the plasma. Thus, primaquine is fairly well absorbed and only a small portion actually reaches the plasma.

Primaquine metabolism occurs at two sites of the molecule: one in the aromatic region at 5- and 6- positions and the other at 8- N aminoalkyl side chain. The first metabolic pathway leads to the formation of 5- hydroxyprimaquine (5-HPQ, 3), 5-hydroxy-demthyl primaquine (5-HDPQ) of the formula (4).



(4)

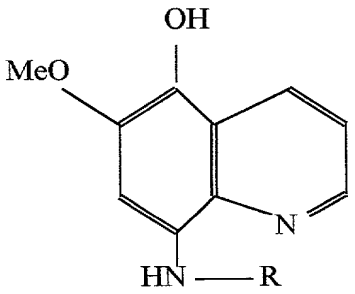
The second pathway originally observed to occur in the microorganisms, affects the 8- N-aminoalkyl chain and results in the formation of N-acetylprimaquine and desamino carboxylic acid of the Formula (12).



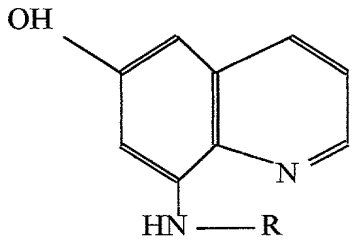
(12)

The carboxylic acid derivative is the major metabolite of primaquine in the human plasma.

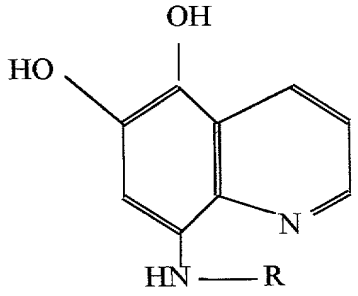
Strother *et al* identified identified metabolites from the urine of primaquine treated dogs as 5-hydroxy-6-methoxy-8-(4-amino-1-methylbutylamino) quinoline of the Formula (3), desmethyl-6-hydroxy-8-(4-amino-1-methylbutylamino) quinoline of the Formula (9) and 5,6-dihydroxy-6-methoxy-8-(4-amino-1-methylbutylamino) quinoline of the Formula (4) shown below: [A. Strother, *et al*, 'Metabolism of *-amonoquinoline antimalarial agents'. **Bulletin of the World Health organisation,** 59, 413-425 (1981)].



(3)

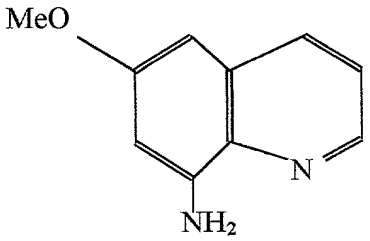


(9)

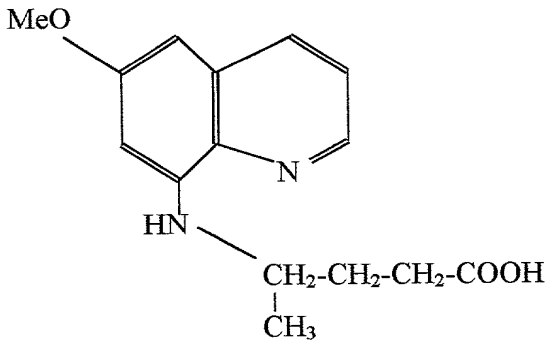


(4)

Among N-dealkylated derivatives of primaquine metabolites were identified as 6-methoxy-8-aminoquinoline of formula (10) [J. D. Baty et al 'The identification of 6-methoxy-8-aminoquinoline as a metabolite of primaquine in Man'. **Biomedical Mass Spectrometry,** 2, 304-306 (1975)] and 8-(3-carboxy-1-methylpropylamino)-6-methoxy quinoline of formula (12) shown below. [J. K. Baker, et al 'HPLC analysis of the metabolism of primaquine and the identification of a New Mammalian Metabolite' **Journal of Chromatography,** 230, 69-77 (1982)].

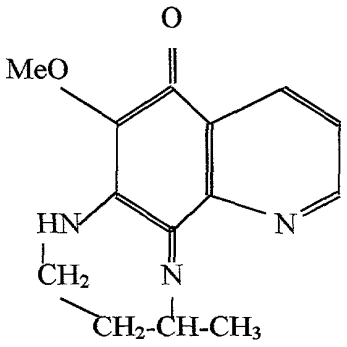


(10)



(12)

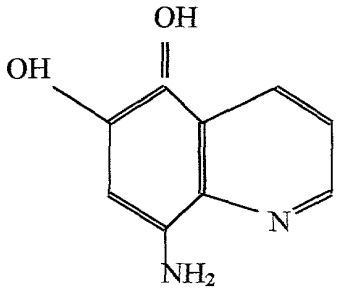
A blue colour metabolite derived from 5-hydroxy-desmethylprimaquine was identified as tricyclic quinomine of formula (8) shown below [A. Strother et al 'Metabolism of Primaquine by various Animal species' in **Primaquine: Pharmacokinetics, Metabolism, Toxicity and Activity,** pp 27-48 (1984), John Wiley & Sons].



(8)

Therapeutic Activity of Primaquine and its Metabolites

Primaquine has blood schizontocidal activities whereas its desmethyl derivative has little. Two 5-OH derivatives of the formula of (3) and (4) shown above are highly active. The quinolines that lack the side chain of 8-position but have merely amino substituents shown in the formula (10) above and formula (11) below have no significant activity.



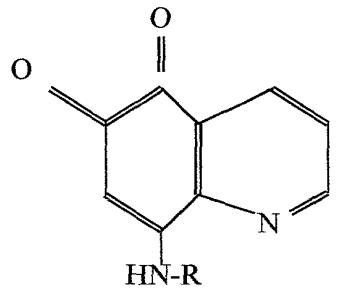
(11)

In marked contrast is the observation that the dealkylated derivatives of the formulae 10 and 11 retain their tissue schizontoidal effect. They are two to three times more active than primaquine.

The direct sporontocidal activity of PQ and of these putative metabolites is poor against the oocysts development when mosquitoes are fed on treated animals that supply the gametocytes. Primaquine is quite inactive as sporontocide when given directly to the insect, but is a very potent gametocytocidal agent.

The 5-hydroxy derivative of the formula (4) of desmethyl primaquine shows only a slight gametocytocidal activity. Desmethyl primaquineof the formula (5) shown below and 5-hydroxy

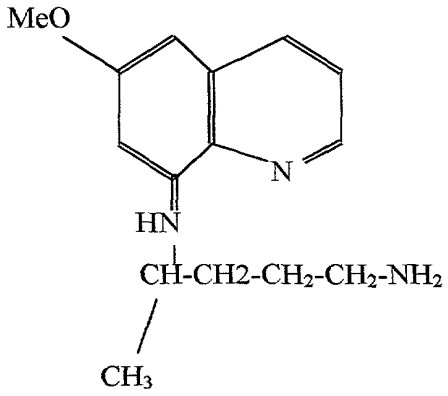
of the formula (3) and carboxylic acid of the formula (12) metabolites of PQ are all inactive. Of particular interest is the observation that two of the quinolines of the formulae (10) and (11) shown above with unsubstituted $-NH_2$ group on 8- position are directly sporontocidal. [W. Peters *et al,* 'The activity of primaquine and its possible metabolites against rodent malaria' **Primaquine: Pharmacokinetics, Metabolism, Toxicity and Activity,** pp 93-101 (1984), John Wiley & Sons].



(5)

Toxicity of Primaquine and its Metabolites:

Primaquine of the formula (2) shown below itself appears to have little oxidant activity even when incubated with G-6PD deficient erythrocytes [I. M. Fraser *et al,* 'Effects of Drugs and Drug Metabolites on Erythrocytes from Normal and Glucose-6-phosphate Dehydrogenase Deficient Individuals', **Annals of the New York Academy of Sciences,** 151, 777-94 (1968)], John Wiley & Sons].



(2)

Whereas 5-hydroxyprimaquine of thr formula (3) and 5, 6-dihydroxy-8-aminoquinoline of the formula (11) cause oxidation of oxyhemoglobin (HbO_2) to methemoglobin (Met Hb) and of reduced glutathione (GSH) [K. A. Fletcher et al, 'The Pharmacokinetics and Biochemical

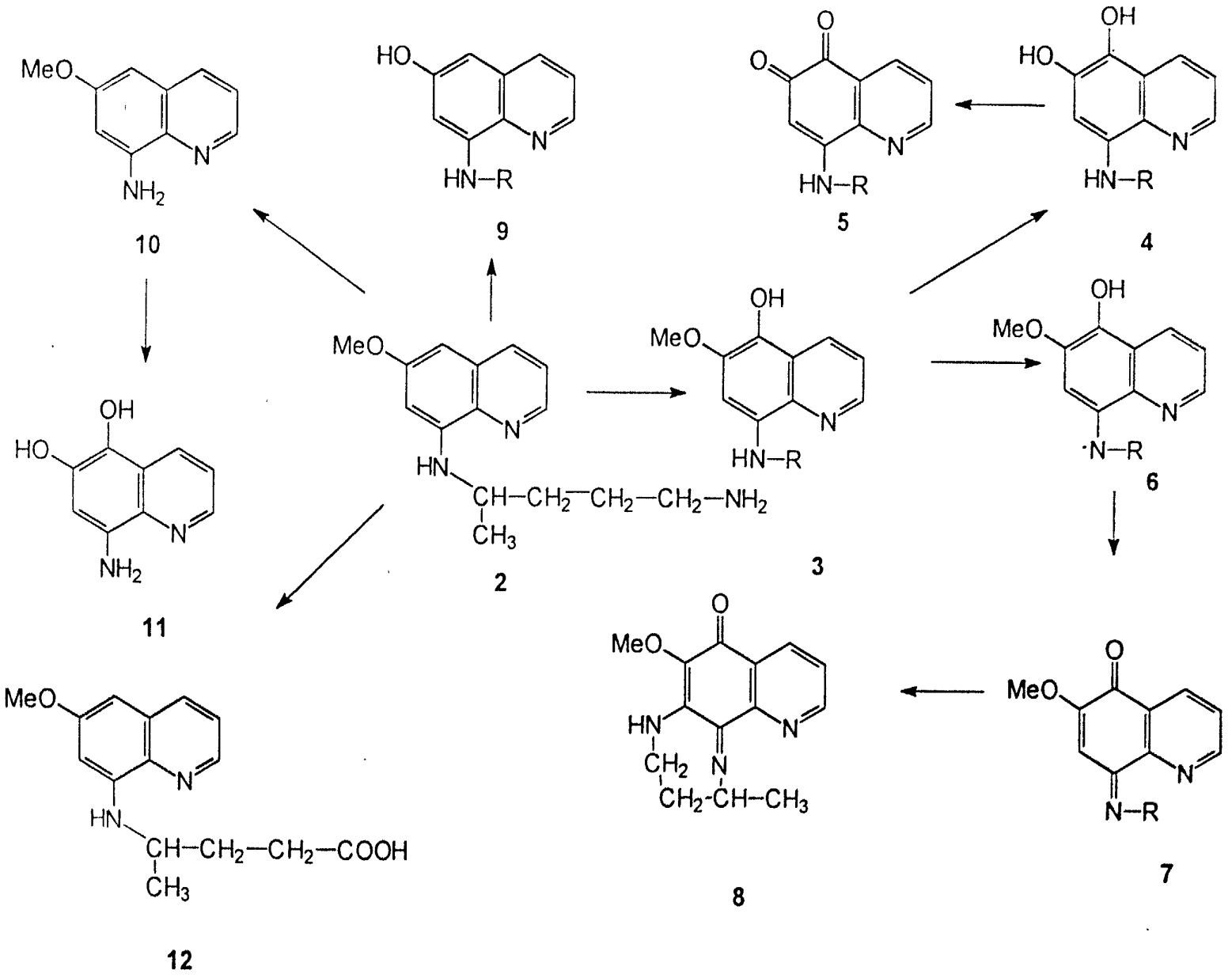
Pharmacology of Primaquine in Rhesus Monkeys and Rats' in **Primaquine: Pharmacokinetics, Metabolism, Toxicity and Activity,** pp 49-63 (1984), John Wiley & Sons].

The carboxylic acid of the formula (12), a major metabolite of primaquine circulating in the plasma, has not shown any antimalarial activity. It is uncertain whether it contributes significantly to the toxicity of primaquine although it does not cause methemoglobin formation *in vitro*. Earlier we reported causal prophylactic activity of primaquine derivative namely N^1 -(3-acetyl-4,5-dihydro-2-furanyl) - N^4 - (6-methoxy-8-quinolinyl) - 1,4-pentanediamine at 3.16 mg/kgX3 doses against sporozoite induces *P. cynomolgi* B. infection in monkeys. The derivative also exerts anti-relapse (radical curative) activity at 1mg/kgX7 days (G. P. Dutta, S. K. Puri, V. C. Pandey, M. Seth, A. P. Bhaduri, S. K. Chatterjee, O. P. Asthana and K. C. Gupta, Tropical Diseases, 286 (1998), G. P. Dutta, S. K. Puri, A. P. Bhaduri and M. Seth, Am. J. Trop. Med. Hyg. 41, 635, (1989). In the derivative, primaquine is substituted at primary amino functionality.

Thus from the above studies, it is obvious that primaquine possesses antimalarial activities such as blood schizontocidal, tissue schizontoidal and gametocytocidal which are also exhibited by its metabolites. Primaquine is even more active than its metabolites. The carboxylic acid of the formula (12) though a major metabolite, is non-functional. The metabolites of primaquine are also responsible for its toxicity. The tricyclic metabolite of the formula (8) is active but less toxic which therefore, suggests the significance of intact side chain. Therefore, if primaquine molecule is manipulated through the side chain possibly toxicity could be modulated. Secondly, primaquine is absorbed and metabolized very fast and as a consequence, oxidative burst accrues very fast. Therefore, its controlled delivery may result in less toxicity. This led us to prepare primaquine prodrug of less toxic profile. Primaquine is of a basic nature with a free amino functionality, which is a point of metabolism for inactive metabolite. We derivatised this amino functionality to enaminone and evaluated its efficacy for gametocytocidal action and methemoglobin toxicity. Enaminones are a functional group for controlled delivery of amino drugs. An enaminone derivative of a physiologically active amine may well show improved transport across biological membranes and allow a high concentration of the amine to be released close to the site of action. This functional group provides resistance towards hydrolytic cleavage at acidic pH as compared to the plain amine. We prepared enaminone derivative of primaquine shown in formula (1) on two accounts. Firstly, it should have slow metabolic degradation through side chain and secondly, compound of enhanced lipophilic character should penetrate better in the tissue, especially in the liver where hypnozoites reside. We therefore, embarked on the preparation of enaminone derivative of formula (1) and the results of its gametocytocidal

effects and its safety profiles are mentioned here. As already mentioned earlier at the beginning, the search for a safe gametocytocidal agent is needed for two reasons, firstly, to block the recurrence of malaria in non-endemic regions where malaria has already been eradicated through vector control methods by individuals visiting endemic regions, and secondly, to block spread of even resistant strains.

Primaquine and its putative metabolites are shown below:



. Primaquine and its putative metabolites

Objects of the invention

The main object of the invention is to provide a new primaquine derivative with the enaminone functionality having gametocytocidal activity and low toxicity for use as a transmission blocker.

Another object of the invention is to provide a new primaquine derivative for facilitating controlled delivery of amino drugs.

It is another object of the invention to provide a primaquine derivative having slow metabolic degradation through the side chain modification.

Another object of the invention is to provide a primaquine derivative with enaminone functional group providing resistance towards hydrolytic cleavage at acidic pH as compared to the plain enamine.

Another object of the invention is to provide a new primaquine derivative with enhanced lipophilic character to facilitate better penetration in the tissue especially in the liver where hypnozoites reside.

It is a further object of the invention to provide a new primaquine derivative with a high therapeutic index ratio in terms of methemoglobin formation.

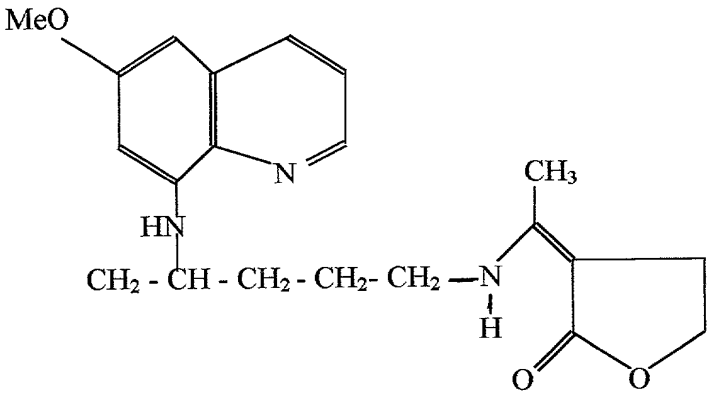
Another object of the invention is to provide a primaquine derivative, which causes oxidation of glutathione (GSH) to a lesser extent.

It is yet another object of the invention to provide a process for the preparation of the novel primaquine derivative of formula (1).

It is a further object of the invention to provide for a method treatment of malaria using primaquine derivative N^1- (3-Ethylidinotetrahydrofuran-2-one)-N^4- (6-methoxy-8-quinolinyl)-1,4-pentanediamine as a gametocytocidal agent.

Detailed description of the invention

The invention provides a method of treatment of malaria using a primaquine derivative of formula (1) shown below with the enaminone functionality having gametocytocidal activity and low toxicity as a transmission blocker. The method comprises administering to the animal, particularly human, infected with malaria, a compound of formula (1) or a pharmaceutical composition containing said compound of formula (1).



(1)

In another embodiment, the invention relates to a method of treatment of malaria using a new primaquine derivative for facilitating controlled delivery of amino drugs.

In a further embodiment, the invention relates to a method of treatment of malaria using a primaquine derivative having slow metabolic degradation through the side chain modification.

In yet another embodiment, the invention relates to a method of treatment of malaria using a primaquine derivative with enaminone functional group providing resistance towards hydrolytic cleavage at acidic pH as compared to the plain enamine.

In another embodiment, the present invention relates to a method of treatment of malaria using a primaquine derivative with enhanced lipophilic character to facilitate better penetration in the tissue especially in the liver where hypnozoites reside.

In another embodiment, the present invention relates to a method of treatment of malaria using a primaquine derivative with a high therapeutic index ratio in terms of methemoglobin formation.

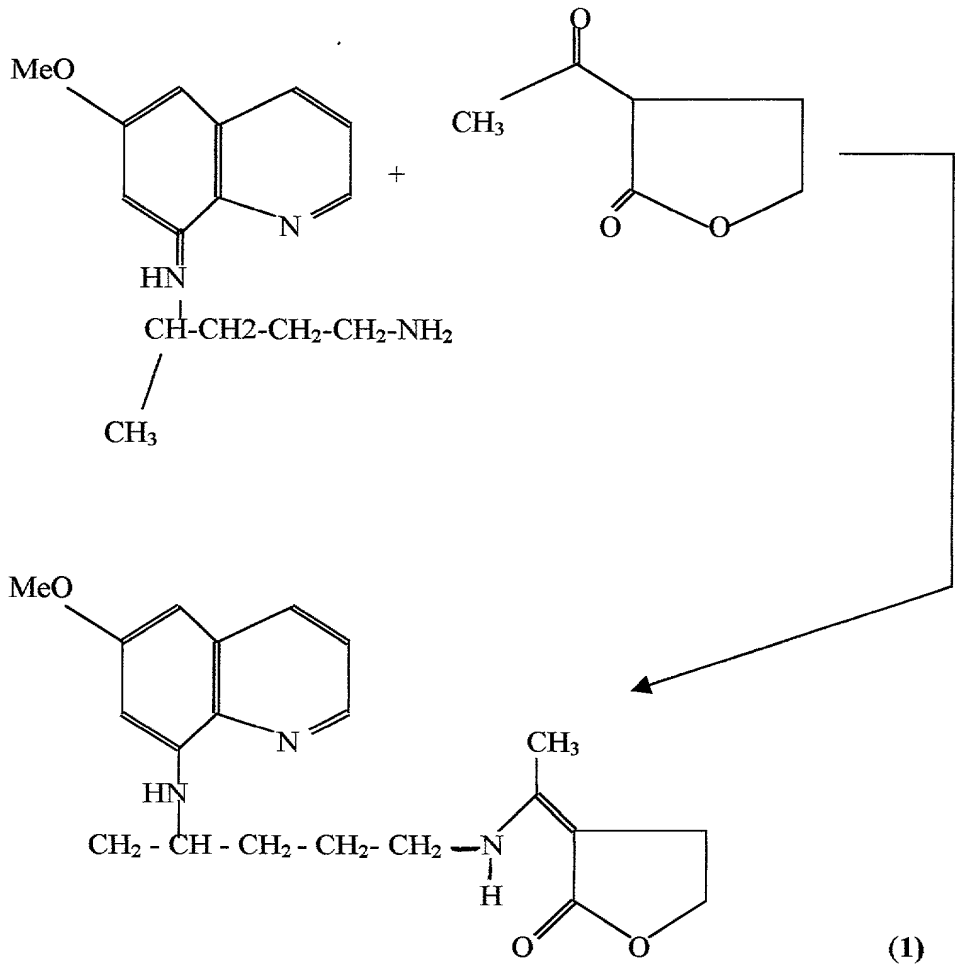
In another embodiment, the present invention relates to a method of treatment of malaria using a primaquine derivative which causes oxidation of glutathione (GSH) to a lesser extent.

In another embodiment, the present invention relates to a process for the preparation of the primaquine derivative of formula (1).

In a further embodiment, the present invention relates to a method of treatment of malaria using primaquine derivative N^1- (3-Ethylidinotetrahydrofuran-2-one)-N^4- (6-methoxy-8-quinolinyl)-1,4-pentanediamine as a gametocytocidal agent.

The process for the preparation of primaquine derivative used in the present comprises the synthesis of enaminone: N^1-(3-ethylidinotetrahydrofuran-2-one)-N^4-(6-methoxy-8-quinolinyl)-1,4-pentanediamine by reaction of 8-(4-amino-1-methylbutylamino)-6-methoxy

quinoline (primaquine) with 3-acetyl-τ-butyrolactone in presence of a base in catalytic amount. The reaction may be represented by the following scheme:



(2)

(1)

The following example illustrates the details of the process of this invention:

N^1- (3-ethylidinotetrahydrofuran-2-one)-N^4- (6-methoxy-8-quinolinyl)-1,4-pentanediamine

A mixture of primaquine base (0.97g, 3.7 mmole) freshly distilled 3-acetyl-r-butyrolactone (1.0g, 7.8 mmole) and a base like piperidine (2-3 drops) were stirred under magnetic stirrer at room temperature. In an hour or so the reaction mixture solidified. The product was titrated in ether and filtered to get the product. It was crystallised from alcoholic solvent like propanol. Yield 0.89g, m.p. 118-120°C.

Gametocytocidal activity:

For the gametocytocidal test, batches of 3-4 day old An. Stephensi were allowed to feed on *P. cynomolgi* infected Rhesus monkeys at appropriate gametocytaemia level. One hour after the control (pretreatment) feeding, compound of formula 1 was administered to the monkeys at 0.63, 1.25, 1.87, 2.5, 3.75 and 5.0 mg/kg in a single dose by oral route. Post-treatment feeding of batches of healthy mosquitoes was done at different times (5-48 hours). Mosquitoes were maintained as 26± 1°C under optimal insectary conditions. The infectivity rate and the oocyst counts were recorded on day 8. Mosquitoes were further maintained in the insectary to record the formation of sporozoites and the absence of sporozoites in some of the batches was also ensured by inoculation of mosquito homogenates into native monkeys.

Results: The gametocytocidal activity of compound of formula (1) was evaluated in 16 Rhesus monkeys and the pre-treatment mosquito infectivity results for these monkeys shows that the oocyst number for different batches ranged from 13.77±9.51 to 125.77± 62,89 and the per cent infectivity varied from 42.55 to 100% (See Table 1). Sequential mosquito feedings on a monkey treated at 0.63 mg/kg dose showed significant reduction in oocyst number and the per cent infectivity at +5h and +24h post-treatment compared to the corresponding control feedings at –1hr. Salivary gland dissections of the mosquitoes from these batches on day 15 showed the presence of sporozoites, thus indicating that oocyst completed normal sporogenic development. No oocysts were observed over the midguts from mosquitoes fed at +48 hr. after drug administration nor were any sporozoites seen in their salivary glands.

Identical results were obtained in the efficacy tests at 1.25 mg/kg in 2/2 monkeys, at 1.87 mg/kg in 2/2 monkeys and at 2.5 mg/kg in 2/3 monkeys. The mosquito batches fed at 4-5 hr. post-treatment showed marked decrease in the oocyst numbers, though these oocysts were able to complete the sporogenic cycle as indicated by the presence of sporozoites in salivary glands on day 15-16. The mosquito batches fed on these monkeys at +24 hr. and +48 hr. did not develop any oocysts nor were any sporozoites demonstrable in their salivary glands.

The oocyst development was completely blocked in the mosquito batches (fed 4-5 hr as well as +24 hr post-treatment) in one of the three monkeys treated at 2.5 mg/kg, 5/5 monkeys treated at 3.75 mg/kg and 3/3 monkeys treated at 5.0 mg/kg dose. Moreover, the salivary gland dissections from these batches carried out between days 14-20 post infective blood meal also did not show any sporozoites. The asexual parasitaemia and gametocytaemia levels for different monkeys is also shown in Table 1. Although the gametocytes were persisting in circulation at +24 hr. and +48 hr. post-treatment, these gametocytes were not infective for An. Stephensi as

indicated by the absence of oocysts. Mosquito batches fed on the vehicle control monkey at –1hr, +24 hr., +48 hr. and +72 hr. showed consistently high per cent infectivity and oocyst number in all the four batches.

Infectivity tests were carried out to ensure that there was no sporozoite development in the mosquito batches found negative for oocysts on day 8 following their feeding on drug treated monkeys. Homogenates of 40-50 mosquitoes each from 11 batches fed on gametocyte carrying monkeys treated with compound 1 at 1.87, 2.5, 3.75 and 5.00 mg base/kg were inoculated into native Rhesus monkeys. None of these 11 monkeys developed potency up to 60 days of observations, indicating complete absence of any viable sporozoites in these batches (Table 1). Similar inoculations made from three pre-treatment (control) batches and one post-treatment batch (from vehicle control) resulted in the development of patent infection in three monkeys on day 9, 10, 10.

A comparision of the mosquito infectivity in batches fed prior to drug administration and at varying intervals after administration of compound of formula (1) has shown drastic reduction of mosquito infectivity and oocyst development. This effect was found to be dose dependant as complete inhibition was obtained at +48 hr. with 1.35-2.50 mg/kg at +24 hr. and the higher doses of 3.75 and 5.00 mg/kg rendered mature gametocytes non-infective to mosquitoes within 4-5 hours. This rapid decline of the mosquito infectivity is attributable to gametocytocidal action of drug. The persisting gametocytes circulating at 24-48 hr. post treatment in compound of formula (1) treated monkeys were non-infective to mosquitoes. Studies with primaquine have shown that 3.16 mg/kg dose produced complete gametocytocidal action at +24 hr. while at 1.00 mg/kg, nearly 98 % loss of infectivity was observed (Table II). The completion of sporogonic cycle in 24-96 hr. old oocysts exposed to the action of compound of formula (1) at 10-50 mg/kg dose indicates absence of sporontocidal/oocysticidal action of the drug (Table III).

Methemoglobin Toxicity Studies

Comparision of Primaquine and Compound of formula (1) in relation to their effect on Methemoglobin

Beagle dogs have been used for obtaining data on the methaemoglobin formation following treatment with compound of formula (1) or primaquine.

Colony bred beagle dogs were maintained in the kennel house of the Institute and fed with a standard diet. Fourteen dogs were divided into five experimental groups as detailed below:

Group I: Three dogs

Primaquine @ 1.0 mg/kg (base) x 7 days

Group II: Three dogs

Primaquine @ 3.0 mg/kg (base) x 7 days

Group III: Three dogs

Compound of formula (1) @ 1.25 mg/kg (base) x 7 days

Group IV: Three dogs

Compound of formula (1) @ 3.75 mg/kg (base0 x 7 days

Primaquine or compound of formula (1) as the case may be was suspended in 0.3 % methyl cellulose solution and administered orally in 10 ml. volume via catheter followed by 5 ml. water to flush the catheter. Treatment was administered once daily for seven doses (day 0-6), the day of the first dose being day 0. The animals were observed for 20-30 minutes for any vomitting. 5 ml. blood was collected from beagle dogs on day 0, 3, 7, 13 and 25 using potassium-oxalate crystals as anticoagulant. All the estimations/tests were conducted on the same day of collection of blood. Methaemoglobin was assayed by the method of Evelyn and Malloy (1938, J. Biol. Chem., 126, 655-662). These values are recorded in Table V. At primaquine antirelapse curative dosage against *P. cynomolgi* in monkeys, (Group I, 1.0 mg/kg), the mean Met-Hb values increased by 3.7 fold on day 7. There was then a gradual decline in Met-Hb values by day 25, but the level was still 2.0 fold the pretreatment level. Primaquine administered at three times the curative dose (Group II, 3.0 mg/kg) showed 10.5 fold increase over the corresponding day 0 value, and the elevated levels again declined after treatment and were 2.5 fold higher than pretreatment values on day 25. Compound 1 at curative dose (Group III: 1.25 mg/kg) only marginally increased the Met-Hb values by 1.7 fold on day 7 and slight increase (2.4 fold) over the pretreatment values on day 25. At the higher dose (Group IV: 3.75 mg/kg) the Met-Hb level on day 7 increased by 3.2 fold and the values declined to 1.8 fold of pretreatment values on day 25. The Vehicle Control Group (Group V) showed marginal fluctuation of Met-Hb level within the normal limits.

Thus on day 7 of the curative dose level, Met-Hb formation was 2.7 fold lower with test compound as compared to primaquine. Likewise, at three times the therapeutic dose, the Met-Hb formation with the test compound was 3.6 fold lower as compared to primaquine.

Reduced Glutathione (GSH) in Human Erythrocytes

Drug induced haemolysis is a serious complication in persons deficient in G-6-PD enzyme. The presence of reduced glutathione (GSH) in erythrocytes control the level of oxidative metabolites. Therefore, drugs, which cause lesser oxidation of GSH level are safe. The

level of reduced glutathione in erythrocytes of healthy and G-6-PD deficient individuals were measured after incubation with PQ and compound of formula (1) and results are mentioned in Tables V and VI. G-6-PD deficiency was detected by the fluorescent spot screening test and confirmed by the enzyme assay method. Heparinised blood samples were collected from each individual and after centrifugation, the packed cells were washed three times with cold saline. One ml. aliquots of washed cells were then incubated with different concentrations of the drugs ranging from 1 to 5 µg/ml base of PQ diphosphate and equivalent doses ranging from 1.25 to 62.5 µg/ml of compound 1 in a water bath at 37°C with occasional agitation for 3 hours. GSH levels were estimated by the method of Bentler *et al* [Improved Method for the Determination of Blood Glutathione, J. Lab. Clin. Med., 61, 882-888 (1963)].

Results:

Mean erythrocyte GSH levels in the controls (without drug) were significantly lower in the G-6-PD deficient individuals (29.5±1.86mg%) as compared to the normals (49.91±4.49 mg%).

Normal erythrocytes exposed to different doses of PQ showed a fall in GSH levels, which reached statistical significance at concentration 10 µg/ml., whereas the same incubated with compound 1 showed significant decrease in GSH levels only at concentration 31.25 µg/ml. (Table V).

At concentration of 25 µg/ml. and 50 µg/ml. of PQ and equivalent doses of compound of formula (1) in G-6-PD deficient erythrocytes, the decrease in GSH level was statistically significant ($P<0.001$) in cow when GSH level compared to GSH levels in other controls. However, the decrease in PQ treated erythrocytes was pronounced as compared to compound of formula (1) treated group, thus showing the higher safety margin of the new compound.

Percentage decrease in GSH levels was more pronounced in normal and G-6-PD deficient erythrocytes treated with PQ as compared to compound of formula (1). Statistically significant decreases were observed at concentrations of 25 µg/ml. and 50 µg/ml. of PQ as compared to the equivalent doses of test compounds in both normal and G-6-PD deficient erythrocytes (Tables V and VI).

Table I: Effect of single dose compound 1 on *P. cynomolgi* B gametocytes as determined by their infectivity to *An. stephensi* mosquitoes.

Dose mg/kg at 0 hr.	Time of mosquito feeding	Para sitaemia/mm^3 Asexual	Gametocytes	Day 7 No. of mosquitoes infected/dissected (% infectivity)		Oocyst record Oocyst No. per gut (Mean ± SD)
0.63	- 1 hr	48816	1728	27/30	(90.0)	86.74 ± 39.2
	+ 5 hr			23/51	(45.1)	10.22 ± 6.8
	+ 24 hr	30024	1404	15/46	(32.61)	2.93 ± 2.4
	+ 48 hr	23220	756	0/24	(0)	Nil
1.25	- 1 hr	126965	1895	34/38	(89.57)	22.35 ± 11.8
	+ 5 hr			12/57	(21.05)	2.17 ± 1.7
	+ 24 hr	103846	1516	0/36	(0)	Nil
	+ 48 hr	15914	109	0/24	(0)	Nil
1.25	- 1 hr	23712	1026	20/47	(42.55)	14.40 ± 7.29
	+ 5 hr			15/70	(21.43)	2.60 ± 1.7
	+ 24 hr	21204	486	0/30	(0)	
1.87	- 1 hr	33602	1166	25/30	(83.33)	28.20 ± 18.9
	+ 4 hr			6/40	(15.00)	1.17 ± 0.4
	+ 24 hr	18020	530	0/27	(0)	Nil
	+ 48 hr	7208	212	0/24	(0)	Nil
1.87	- 1 hr	61560	1026	23/25	(92.0)	80.69 ± 35.7
	+ 4 hr			18/31	(58.06)	13.00 ± 12.3
	+ 24 hr	42180	798	0/38	(0)	Nil**
	+ 48 hr	5130	228	0/21	(0)	Nil
2.50	- 1 hr	33578	1442	36/46	(78.26)	13.72 ± 9.5
	+ 4 hr			20/33	(60.61)	2.90 ± 2.2
	+ 24 hr	45320	927	0/29	(0)	Nil
	+ 48 hr	18025	206	0/21	(0)	Nil
2.50	- 1 hr	135464	4130	26/28	(92.86)	125.77 ± 62.8
	+ 5 hr			11/30	(36.67)	4.64 ± 2.8
	+ 24 hr	96642	2478	0/30	(0)	Nil
2.50	- 1 hr	38081	2147	29/37	(78.38)	55.79 ± 41.0
	+ 5 hr			0/33	(0)	Nil**
	+ 24 hr	31075	1243	0/44	(0)	Nil**
3.75	- 1 hr	55728	1296	26/27	(96.30)	22.35 ± 15.8
	+ 4 hr			0/25	(0)	Nil
	+ 24 hr	55808	540	0/28	(0)	Nil

3.75	- 1 hr	45796	1070	15/22 (68.18)	22.00 ± 16.3
	+ 4 hr			0/21 (0)	Nil
	+ 24 hr	25894	535	0/21 (0)	Nil
3.75	- 1 hr	68320	2318	33/40 (82.50)	60.64 ± 35.4
	+ 5 hr			0/30 (0)	Nil**
	+ 24 hr	26108	366	0/30 (0)	Nil**
3.75	- 1 hr	48336	954	22/22 (100.0)	
	+ 4 hr			0/41 (0)	
	+ 24 hr	65084	1696	0/27 (0)	

Table II : Gametocytocidal Activity of Primaquine

Dose mg/kg at 0 hr	Time of mosquito feeding	Parasitaemia/mm^3		Day 7 oocyst record	
		Asexual	Gametocytes	No.of mosquitoes infected/dissected (%infectivity)	oocyst no. per gut (Mean±SD)
1.00mg/kg	-1 hr	36166	1428	32/40 (80.0)	17.13±10.0
	+5 hr			32/44 (72.7)	13.69± 7.2
	+24hr	28048	526	0/55 (0)	Nil
	+48hr	15332	234	0/40 (0)	Nil
1.00mg/kg	-1 hr	42394	5152	25/34 (72.53)	37.14±16.6
	+5 hr			36/46 (78.26)	34.08±14.7
	+24hr	26832	3256	3/45 (6.67)	2.17± 1.7
	+48hr	12140	635	0/40 (0)	Nil
3.16mg/kg	-1 hr	29680	1230	37/51 (72.55)	57.59±31.0
	+5 hr			0/53 (0)	Nil
	+24hr	23112	749	0/33 (0)	Nil
3.16mg/kg	-1 hr	16824	1026	20/47 (42.55)	24.4 ± 7.2
	+5 hr			15/46 (32.61)	2.6±1.76
	+24hr	21204	670	0/43 (0)	Nil

Table III : Effect of Compound 1 on developing oocysts of *P. cynomolgi* *An. stephensi* mosquitoes

Age of Infection in mosquitoes	Mosquito feeding on drug treated*/ control monkey	Day 8 oocyst record	
		No. of mosquitoes infected/dissected (%infectivity)	oocyst number gut (Mean±SD)
24 hr	10mg/kg	17/20 (85.00)	144.47±60.35
	Control	15/18 (83.33)	133.33±62.30
	50mg/kg	23/27 (85.19)	67.00±43.58
	Control	29/36 (80.56)	66.00±43.48
48 hr	10mg/kg	20/20 (100.0)	133.20±96.22
	Control	19/21 (90.48)	124.05±65.85
	50mg/kg	26/33 (78.79)	46.15±36.70
	Control	28/34 (82.35)	42.57±35.27
72 hr	10mg/kg	22/25 (88.00)	20.36±17.81
	Control	23/28 (82.14)	26.83±19.00
	50mg/kg	25/29 (86.21)	27.16±20.60
	Control	22/32 (68.75)	26.59±22.05
96 hr	50mg/kg	18/26 (69.23)	40.33±27.38
	Control	19/25 (76.00)	47.42±28.46

* Mosquitoes with 24-96 hr old oocysts were allowed to engorge blood from naive monkey administered compound 1at -7 hr of the mosquito feeding

** Patent infection developed on days 9-10 in naive monkeys upon inoculation of 10 mosquitoes' homogenates.

Table IV: Methaemoglobin levels (g%) in Beagle dogs after treatment with Primaquine and compound 1.

Group	Treatment	Day 0	Day 3	Day 7	Day 13	Day 25
1.	Primaquine 1.0 mg/kg	0.65 ± 0.03	0.85 ± 0.13	02.39 ± 0.23	1.98 ± 0.34	1.33 ± 0.0
2.	Primaquine 1.0 mg/kg	0.74 ± 0.07	1.94 ± 0.33	7.81 ± 1.48	5.51 ± 1.03	1.86 ±0.0
3.	Compound 1 1.25 mg/kg	0.53 ± 0.11	0.87 ± 0.17	0.89 ± 0.29	1.04 ± 0.07	1.26 ± 0.19
4.	Compound 1 3.75 mg/kg	0.66 ± 0.15	1.0 ± 0.19	2.14 ± 0.89	1.66 ± 0.52	1.18 ± 0.14
5.	Compound 1 1.0 mg/kg	0.64 ± 0.09	0.46 ± 0.09	0.74 ± 0.01	0.65 ± 0.10	0.83 ± 0.0

Day 0 = Start of drug treatment
Day 3 = After three doses
Day 7 = 1 day after last dose of drug
Day 13 = 7 days after last dose of drug
Day 25 = 19 days after last dose of drug

Table V: GSH levels in normal erythrocytes with different doses of primaquine and equivalent doses of compound 1.

Primaquine		Compound 1	
Dose (µg/ml)	GSH (mg%) (Mean ± SE)	Dose (µg/ml)	GSH (mg%) Mean ± SE
Control (No drug)	49.91 ± 4.49	Control (No drug)	49.91 ± 4.49
1.00	43.50 ± 5.70	1.25	44.08 ± 5.80
5.00	39.00 ± 6.16	6.25	42.50 ± 5.85
10.00	29.67 ± 6.49	12.50	38.25 ± 5.68
25.00	19.42 ± 2.83	31.25	31.00 ± 5.15*
50.00	10.37 ± 1.57	62.50	32.75 ± 5.39*

* Comparison of equivalent doses of compound 1 with primaquine * P<0.05** P<0.01

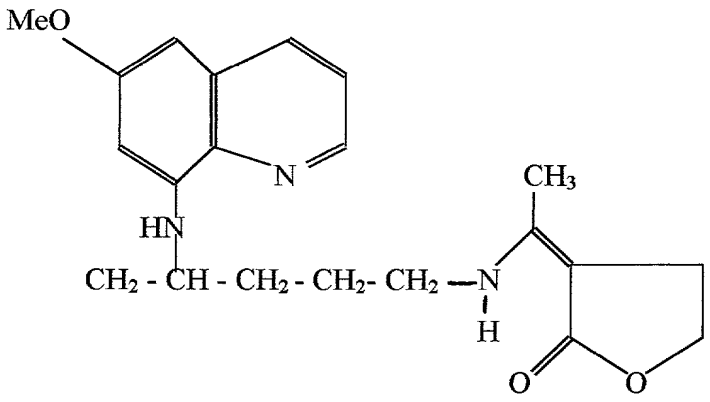
Table VI: GSH levels in G-6-PD deficient erythrocytes with different doses of primaquine and equivalent doses of compound 1.

Primaquine		Compound 1	
Dose (µg/ml)	GSH (mg%) (Mean ± SE)	Dose (µg/ml)	GSH (mg%) Mean ± SE
Control (No drug)	29.50 ± 1.86	Control (No drug)	29.50 ± 1.86
1.00	25.75 ± 2.17	1.25	26.04 ± 2.20
5.00	19.17 ± 1.50	6.25	23.42 ± 1.66
10.00	14.83 ± 1.89	12.50	20.00 ± 1.73
25.00	10.50 ± 1.52	31.25	17.17 ± 1.81*
50.00	9.00 ± 1.94	62.50	16.62 ± 1.84*

* P<0.05. Comparison of compound 1 with primaquine

We claim:

1. A method of treatment of malaria in animals, particularly humans which comprises administering to said animals, particularly humans, a primaquine derivative of formula 1



(1)

or a pharmaceutical composition containing said primaquine derivative of formula (1), wherein the enaminone functionality of said derivative has gametocytocidal activity and low toxicity and is used as a transmission blocker.

2. A method as claimed in claim 1, wherein said derivative facilitates controlled delivery of amino drugs.
3. A method as claimed in claim 1 wherein said derivative has slow metabolic degradation through the side chain modification.
4. A method as claimed in claim 1, wherein said derivative has a enaminone functional group to provide resistance towards hydrolytic cleavage at acidic pH as compared to the plain enamine.
5. A method as claimed in claim 1, wherein said derivative has enhanced lipophilic character to facilitate better penetration in the tissue especially in the liver where hypnozoites reside.
6. A method as claimed in claim 1, wherein said derivative has a high therapeutic index ratio in terms of methaemoglobin formation.
7. A method as claimed in claim 1, wherein said derivative causes substantially lesser oxidation of glutathione (GSH).

8. A method of treatment of malaria using a primaquine derivative N^1- (3-ethylidinotetrahydrofuran-2-one)-N^4- (6-methoxy-8-quinolinyl)-1,4-pentanediamine as a gametocytocidal agent.

9. A process for the preparation of primaquine derivative of formula 1 which comprises reacting 8-(4-amino-1-methylbutylamino)-6-methoxy quinoline (Primaquine) with 3-acetyl-τ-butyrolactone in presence of a base in catalytic amount to provide the required product.

10. A process as claimed in claim 9 wherein said compound of formula (1) is enaminone N1 - (3-ethylidinotetrahydrofuran-2-one) - N4 - (6-methoxy -8 -quinolinyl) -1,4 - pentanediamine

ABSTRACT

The present invention a novel use of primaquine derivative N^1 - (3-ethylidinotetrahydrofuran-2-one)-N^4 - (6-methoxy-8-quinolinyl)-1,4-pentanediamine in the treatment and contolling the spread of malaria. In particular, the present invention discloses a method of treatment of malaria by the use of primaquine derivative N^1 - (3-ethylidinotetrahydrofuran-2-one)-N^4 - (6-methoxy-8-quinolinyl)-1,4-pentanediamine as a gametocytocidal agent.